Breast Cancer Treatment

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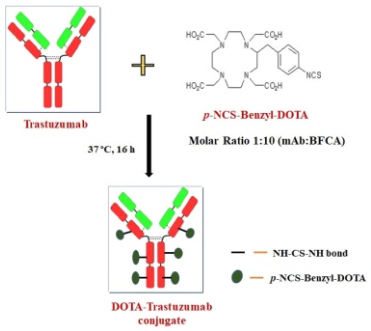
Development and evaluation of [^{177}Lu]Lu-labeled-Trastuzumab for Radioimmunotherapy of Cancer Patients Suffering with HER2 Positive Breast Cancer

Mohini Guleria*[1,2], Jeyachitra Amirdhanayagam[2], Rohit Sharma[1] and Tapas Das[1,2]
[1]*Radiopharmaceuticals Division, Bhabha Atomic Research Centre (BARC), Trombay-400085, INDIA*
[2]*Homi Bhabha National Institute, Anushakti Nagar, Mumbai-400094, INDIA*



Pictorial depiction of conjugation reaction between Trastuzumab and p-NCS-benzyl-DOTA

ABSTRACT

Trastuzumab, an FDA-approved humanized monoclonal antibody, is utilized in the treatment of HER2-positive breast cancer. This study aims to optimize a freeze-dried formulation of DOTA-Trastuzumab conjugate for the preparation of patient dose of [^{177}Lu]Lu-Trastuzumab, intended for radioimmunotherapy of breast cancer. The conventional formulation process for [^{177}Lu]Lu-Trastuzumab is time-consuming and impractical for routine preparation in hospital radiopharmacies. To address this, a pre-synthesized DOTA-Trastuzumab conjugate in freeze-dried form was prepared after the addition of carefully optimized radioprotectant and cryoprotectant. The final radiochemical purity of [^{177}Lu]Lu-DOTA-Trastuzumab, prepared using the freeze-dried kit, was found to be >95%. To ensure the reproducibility of the process, six consecutive batches of the freeze-dried formulation are prepared and evaluated. Preliminary clinical evaluation of [^{177}Lu]Lu-Trastuzumab was performed in patients diagnosed with HER2-positive breast cancer.

KEYWORDS: *[^{177}Lu]Lu, [^{177}Lu]Lu-Trastuzumab, DOTA-Trastuzumab, Freeze-dried kit, Radioimmunotherapy*

Introduction

Trastuzumab, a monoclonal antibody (IgG) has been approved by US-FDA for immunotherapy of the HER2 positive breast cancer in 1998. However, in many cases, conventional immunotherapy involving Trastuzumab suffers from various issues, such as cardiotoxicity and other related complications [1,2]. Receptor heterogeneity and receptor down-regulation during the course of immunotherapy are additional factors resulting in low response of the breast cancer patients during Trastuzumab therapy [1,2]. To circumvent these issues, instead of using 'cold' (non-radioactive) antibody, the use of Trastuzumab in radiolabeled form has been envisaged, wherein the antibody is radiolabeled with a suitable radionuclide for exerting therapeutic effects. The aforementioned technique is termed as 'radioimmunotherapy' (RIT), where unlike immunotherapy, the associated radionuclide is responsible for therapeutic efficacy whereas the monoclonal antibody acts as the targeting vector only [3-5]. The application of [^{177}Lu]Lu-DOTA-Trastuzumab is currently being explored clinically for diagnosis and radionuclidic therapy of patients suffering from HER2 positive breast cancer [3-5]. Lutetium-177 [$E_{\beta max}$= 0.497 MeV] is a therapeutic radionuclide suitable for development of radiolabeled antibodies especially due to its long half-life ($T_{1/2}$ = 6.73 d) which matches well with that of the biological half-life of monoclonal antibodies. Given recent interest in the application of radiolabeled antibodies in cancer care, convenient and simple formulation of radiolabeled antibodies at hospital radiopharmacies is highly desirable. Therefore, an attempt has been made to formulate a robust 'freeze-dried' Trastuzumab-kit which will enable the preparation of patient doses of ^{177}Lu-labeled Trastuzumab at hospital radiopharmacy in a simple and convenient manner. In the present work, systematic optimization of the kit constituents was carried out to arrive at the formulation which consistently give high and reproducible radiolabeling yields for [^{177}Lu]Lu-DOTA-Trastuzumab using medium specific activity ^{177}Lu [555-740 MBq/µg].

*Author for Correspondence: Mohini Guleria
E-mail: mohini@barc.gov.in

Materials and Methods

Conjugation of Trastuzumab with *p*-NCS-benzyl-DOTA (subsequently referred as DOTA) was performed by following the reported method [5]. The average number of DOTA-units attached per Trastuzumab molecule was analysed by two different methods viz. UV-Vis spectrophotometry as well as MALDI-TOF mass spectrometry. The purified DOTA-Trastuzumab conjugate was also analysed for protein content by employing Bradford protein assay. The formulation of freeze-dried kit of DOTA-Trastuzumab was carried out by following a procedure mentioned as: A stock solution 4.0 mL containing 32 mg of DOTA-Trastuzumab in 0.2 M NaOAc buffer (pH 5.0) was used. The other stock solutions (aqueous) utilized were of sucrose (50.0 mg/mL), ascorbic acid (50.0 mg/0.5 mL) and 0.2 N NaOH (10 mL). Aliquots of 375 µL (3.0 mg) of DOTA-Trastuzumab conjugate, 100 µL of sucrose (5.0 mg), 50 µL of ascorbic acid (5.0 mg), 0.2 N NaOH (1.0 mL) and 0.2 N NaOAc buffer (1.0 mL) were withdrawn and added to each sterile glass vial. The vials were lyophilized and stored at 4°C till further use or till shelf-life of kit (6 months).

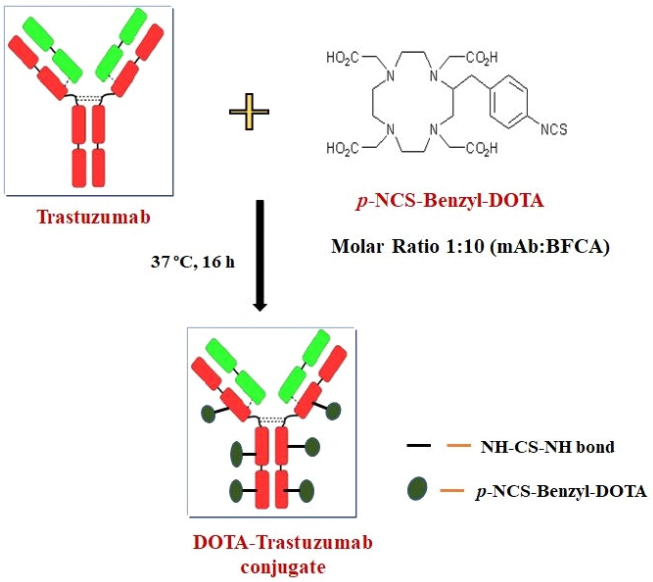


Fig.1: Pictorial depiction of conjugation reaction between Trastuzumab and p-NCS-benzyl-DOTA.

For radiolabeling, the freeze-dried DOTA-Trastuzumab formulation was reconstituted with 0.5 mL of deionized water followed by addition of 100-200 µL (1.94-2.40 GBq) of [^{177}Lu]$LuCl_3$ (in 0.01 M HCl). The reaction mixture was incubated at 37^{0}C for 30 minutes. Radiochemical purity of [^{177}Lu]Lu-DOTA-Trastuzumab was confirmed using paper chromatography (PC) and High Performance Liquid Chromatography (HPLC). PC was performed with 0.01 M sodium citrate buffer (pH 5.0) as mobile phase, while HPLC utilized a size-exclusion column (TSK Gel G3000SWXL) with isocratic elution using 0.05 M phosphate buffer (pH 7.0) containing 0.05% NaN_3 as the mobile phase.

Post-radiolabeling, [^{177}Lu]Lu-DOTA-Trastuzumab was evaluated in three different cancer cell lines (SK-OV-3, SK-BR-3 and MDA-MB-231) for determination of binding and specificity towards HER2 receptors. Cells were treated with [^{177}Lu]Lu-DOTA-Trastuzumab [5 MBq, 50 nM] for 2 h followed by washing with ice-cold 0.05 M PBS (pH 7.0) and solubilization with of 1N NaOH. The extracted cells were centrifuged, supernatant was removed and the activity associated with the cells was counted. Inhibition studies were carried out under same conditions by incubating the cancer cells with [^{177}Lu]Lu-DOTA-Trastuzumab and cold/non-radioactive Trastuzumab (3.3 µM).

Several physico-chemical studies were performed before [^{177}Lu]Lu-DOTA-Trastuzumab, formulated using DOTA-Trastuzumab kit, was released for further experimentation/use. Bio-evaluation of [^{177}Lu]Lu-DOTA-Trastuzumab was performed in healthy Swiss mice (n=3) at different post-administration (p.i.) time points namely 1, 2, 5 and 7 d as well as in SK-OV-3 xenografted SCID mice at 48 h p.i. Post-administration, the animals were sacrificed, dissected and activity associated with various organs was determined as percentage injected activity per gram of organ (%IA/g of organ).

[^{177}Lu]Lu-DOTA-Trastuzumab prepared using in-house optimized freeze-dried kit of DOTA-Trastuzumab was utilized for limited clinical evaluation in patients suffering with HER2 positive breast cancer.

Results and Discussion

DOTA-Trastuzumab conjugate was synthesized by conjugating -NH_2 group of lysine amino acid in Trastuzumab with isothiocyanate group of DOTA derivative employing 1:10 molar ratio (Fig.1). Mass analyses revealed the average number of BFCA molecules attached per Trastuzumab as 6.0±1.1, whereas using the UV-Vis spectrophotometric assay the same was determined to be 6.2±0.8 (Fig.1).

Optimized freeze-dried Trastuzumab kit comprised 3.0 mg DOTA-Trastuzumab, 5.0 mg sucrose, 5.0 mg ascorbic acid, 8 mg NaOH and 16.5 mg NaOAc. Considering that minimum radiochemical purity of [^{177}Lu]Lu-DOTA-Trastuzumab should be ~95% for clinical application, it was observed that maximum 2.22 GBq of ^{177}Lu could be added in the kit vial, when ^{177}Lu having specific activity of 555 MBq/µg was used for the formulation of the radiolabeled agent.

PC and HPLC were used for the determining of RCP of [^{177}Lu]Lu-DOTA-Trastuzumab formulated using kit. In PC, [^{177}Lu]Lu-DOTA-Trastuzumab remained at the origin (R_f = 0.0-0.1) whereas free ^{177}Lu moved to the solvent front R_f = 1.0). In HPLC, [^{177}Lu]Lu-DOTA-Trastuzumab exhibited a retention time (R_t) of 14.5±0.5 min whereas uncomplexed ^{177}Lu eluted from the column at 21.2±0.9 min. The percentage binding [^{177}Lu]Lu-DOTA-Trastuzumab in SK-OV-3 and SK-BR-3 cells ranged from 14.6±2.1 to 23.0±1.4 and 19.5±0.9 to 32.0±7.2, respectively; whereas the same in MDA-MB-231 cells was observed to vary from 3.2±1.4 to 4.6±1.0. In addition to a lower binding in negative control, a significant decrease in the % binding of [^{177}Lu]Lu-DOTA-Trastuzumab in HER2 positive cancer cells in presence of excess of unmodified Trastuzumab was also observed. As a part of additional quality control studies, visual examination of [^{177}Lu]Lu-DOTA-Trastuzumab showed the formulation as a clear, colorless and transparent solution. The pH of the final preparation was observed to be between 5.0 and 6.0.

Bio-evaluation studies revealed considerable uptake and prolonged retention of the radiolabeled agent in blood (21.47±4.81, 17.06±0.79, 15.55±2.84 and 12.32±3.94%IA/g at 1, 2, 5 and 7 d p.i., respectively). Low accumulation and retention of the radiotracer was observed in majority of the organs except in liver and intestine. Bio-distribution studies performed in SCID mice having SK-OV-3 xenografted tumor showed tumor uptake of 9.07±2.60 % IA/g at 48 h p.i. which indicated the *ex-vivo* tumor targeting potential of [^{177}Lu]Lu-DOTA-Trastuzumab formulated by using kit.

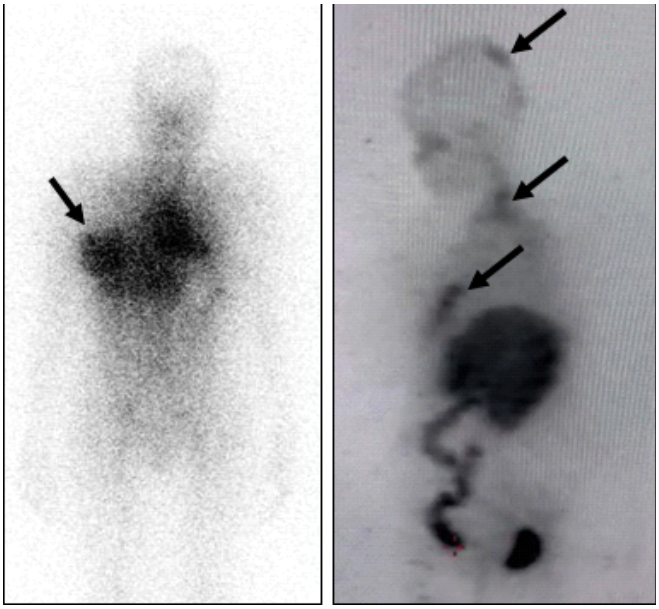


Fig.2: SPECT scans of two patients depicting the accumulation of [^{177}Lu]Lu-DOTA-Trastuzumab (shown with arrows) in cancerous lesions (Image courtesy: Dr. Venkatesh Rangarajan, TMH Parel and Dr. Nandini Pandit, JIPMER, Puducherry).

During clinical evaluation, [^{177}Lu]Lu-DOTA-Trastuzumab exhibited accumulation in cancerous lesions thereby showcasing the retention of its targeting efficacy post-functional modification and radiolabeling procedures (Fig.2).

Conclusion

The formulation of DOTA-Trastuzumab as a lyophilized kit, suitable for the formulation of patient dose of [^{177}Lu]Lu-DOTA-Trastuzumab, has been standardized. The kit was used for the formulation of patient dose of [^{177}Lu]Lu-DOTA-Trastuzumab with high RCP in a reproducible manner. Availability of DOTA-Trastuzumab freeze-dried kits will help in easy, convenient and consistent formulation of [^{177}Lu]Lu-DOTA-Trastuzumab patient doses in the hospital radiopharmacies.

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